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REHABILITATION FROM NEONATAL HYPOTHYROIDISM: SPONTANEOUS MOTOR ACTIVITY, EXPLORATORY BEHAVIOR, AVOIDANCE LEARNING AND RESPONSES OF PITUITARY – THYROID AXIS TO STRESS IN MALE RATS*

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SUMMARY

Long-Evans male rats were made hypothyroid from birth by the addition of 6-*N*-propylthiouracil (PTU) to their drinking water (0.1%). A group of animals was rehabilitated beginning at postnatal day 25 by withdrawal of the PTU from the drinking water. Subsequently, the rats were tested for a variety of behavioral tasks. Serum concentrations of thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) were determined by radioimmunoassay. At 50 days of age, PTU-treated rats had non-detectable levels of T4 but an eight-fold increase of TSH. In 50-day-old, neonatally hypothyroid but rehabilitated rats, serum TSH and T3 were normal, although T4 was still significantly lower. At 90 days of age, basal levels of TSH and thyroid hormones were normal in the rehabilitated rats, but thyroid hormone secretion in response to various types of neural stress was markedly altered. Comparison of passive avoidance learning revealed no significant alteration in the memory retention of either PTU-treated or rehabilitated animals. The 50-day-old, rehabilitated rats showed increased locomotor activity both in running-wheel and in hole-board tests; this hyperactivity, though markedly reduced, still persisted at day 90. In the early phase of rehabilitation (50 days of age), decreases in exploratory activity and lack of habituation occurred with the hole-board test; by the late phase of rehabilitation (90 days of age) these behavioral parameters had become normal. These results suggest generally longer periods of plasticity of the brain and better prospects for rehabilitation from neonatal cretinoid retardation than commonly believed. Specifically, the pituitary – thyroid system and neural mechanisms integrating adaptive behavior possess considerable capacity for spontaneous recovery from hypothyroidism; certain types of altered neuroendocrine and behavioral responses appear to be less amenable to rehabilitation or require longer periods for complete rehabilitation.

INTRODUCTION

IT IS GENERALLY believed that the first 3 weeks of postnatal life in the rat are critical periods for the development of the nervous system (Timiras, 1972). Thyroid deficiency during this period severely impairs growth and development of the brain and spinal cord as well impairing behavior (Balazs *et al.*, 1971; Eayrs, 1966; Ford & Cramer, 1977; Meisami, 1983; Grave, 1977; Legrande, 1983; Timiras & Luckock, 1974). In earlier studies, to investigate the permanence or reversibility of the effects of neonatal thyroid hormone deficiency on behavior, hypothyroid rats were given replacement therapy with

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thyroxine (T4) or triodothyronine (T3) at various ages and subsequently assessed as adults for somatic and behavioral competence (Eayrs, 1966; Eayrs & Levine, 1963). In other experiments, hypothyroid rats receiving a goitrogen for thyroid suppression were allowed to recover spontaneously by withdrawal of the goitrogen and were later assessed at different adult ages with a variety of behavioral tests (Eayrs, 1966). Thus, it was shown that replacement therapy with thyroid hormone restored some of the behavioral and cognitive responses (e.g., learning of the conditioned avoidance response) but not others (e.g., performance in complex maze tasks such as those examined in the Hebbs – William test). Essman *et al.* (1968), Davenport & Dorcey (1972), and Davenport & Hennies (1976) concluded that early postnatal thyroid deficiency resulted in lasting deficits in learning capacity and motivational and metabolic factors, even when overall somatic retardation was only temporary, implying the existence of critical periods for the effects of thyroid hormones on behavioral development. In the majority of these earlier studies (prior to the advent of radioimmunological techniques), serum concentrations of thyroid-stimulating hormone (TSH), T4, and T3 were not monitored (Eayrs 1961; 1964; Hamburgh *et al.*, 1964, Essman *et al.*, 1968; Davenport & Hennies, 1976). Furthermore, the behavioral abilities and learning performance of the animals were tested in adulthood but not during the earlier and more immediate periods of rehabilitation. No information was provided on the dynamics of the brain – pituitary – thyroid axis in the rehabilitated rats, in particular with regard to responses to stressful stimuli.

The efficiency and rapidity of rehabilitation after replacement therapy is of particular significance in the treatment of congenital hypothyroidism in humans. With the increasing prevalence of screening for neonatal hypothyroidism, we are confronted with the need to restore as complete a pattern of growth and development as possible. Thus far, studies in hypothyroid infants and children indicate that some functions (e.g., gross motor development, verbal intelligence, social competence) are restored completely and others (e.g., fine motor and perceptual development) only partially, while some (e.g., temperamental disturbances) worsen (Glorieux *et al.*, 1983; Wolter *et al.*, 1979).

We herein report our studies of several behavioral parameters, such as spontaneous motor activity, exploratory behavior, and passive avoidance learning, in 50- and 90-day old rats rehabilitated from neonatal hypothyroidism. These rats had been deprived of thyroid hormones by the administration of propylthiouracil (PTU) in the drinking water of the mother and pups for the first 25 days of life and then allowed to recover spontaneously from hypothyroidism by termination of the PTU treatment. The responses of these rehabilitated rats then were compared with those of hypothyroid and normal rats. In addition, the serum levels of T4, T3, and TSH were measured in the normal, hypothyroid, and rehabilitated groups. Finally the functional state of the brain – pituitary – thyroid axis in the rehabilitated rats was tested by comparing with normal rats the ability to secrete thyroid hormones in response to neural stress.

METHODS

Animals

The experiments were performed on Long-Evans rats bred in our animal housing facilities. Pregnant female rats were housed in individual cages with *ad libitum* food (Purina rat chow) and water in a temperature controlled environment (23 ± 1°C) on a 14:10 hr light:dark schedule (lights on from 0500 hr to 1900 hr).

Altogether, 90 pregnant rats were used in the experiments, the animals being divided equally between the three groups of normal controls, hypothyroid and hypothyroid-rehabilitated animals. From the second week of pregnancy, females were handled daily to habituate them to the experimental procedures and to enhance survival of the pups, particularly in the hypothyroid groups. After delivery (designated postnatal day 1), the pups from various litters were combined. Each newly formed litter was limited to eight male pups. In all the experiments, a total number of 158 untreated control, 114 hypothyroid, and 135 neonatally hypothyroid but rehabilitated rats was used. The experimental rats were made hypothyroid by addition of 6-*N*-propylthiouracil (PTU) (Sigma) to the drinking water (0.1%) from the day of parturition. Saccharin (1 mg/100 ml) was added to sweeten the bitter taste of the PTU water. Contol (normal) rats also received saccharin. Water intake of the mothers and body weight of the pups were measured daily. From the 17th postnatal day, powdered Purina Rat Chow was freely available for the hypothyroid pups.

Administration of PTU to the lactating rat reversibly inhibits both maternal and infant thyroid function, interrupting the uptake of iodine by the thyroid gland and suppressing both the activity of the thyroid peroxidase enzyme and the peripheral conversion of T4 to T3 (Davidson *et al.*, 1978; Shiroozu *et al.*, 1983). Beginning with day 25, a group of hypothyroid rats was allowed to rehabilitate by withdrawal of the PTU from the drinking water.

Mortality was negligible in the control groups, higher among the hypothyroid rats (about 10–20% in the preweaning period and more in the postweaning phase), and near normal in the rehabilitated animals.

Behavioral tests

Though the main purpose of the present study was the assessment of the extent of behavioral recovery from neonatal hypothyroidism, we chose to study the behavior of the hypothyroid rats as well. Our choice of the 50-day hypothyroid rats for this purpose (see below) was dictated by our need to compare the behavior of the rehabilitated animals not only with the normal animals but also with the hypothyroid animals of the same age, so that we would be able to assess the extent of compensation of the behavior as well as its degree of recovery. Also, in general, the behavior of hypothyroid rats does not change appreciably between day 25 (weaning) to days 42–50, which is the age period when behavior was examined. The behavior of the 90-day-old hypothyroid animals could not be studied, because very few survived up to this age without serious illness. Indeed, in the behavioral tests detailed below, we used only those hypothyroid rats which showed no overt signs of severe illness, weakness, or malnutrition, exhibiting reasonable activity and vitality, notwithstanding their cretinoid condition.

Spontaneous motor activity of 42- to 50-day-old (referred to as 50-day) control, hypothyroid, and rehabilitated, as well as 90- to 100-day-old (referred to as 90-day) normal and rehabilitated rats was measured in a running-wheel apparatus in a sound-attenuated, temperature- and light-controlled environment. Rats were allowed to habituate to the experimental cages for 4 days; thereafter, their 24-hr motor activity was measured for 4 consecutive days. The electronic counter of the running-wheel cages was connected to a four channel polygraph (Harvard type 2120). The chart record provided information on the daily distribution of running activity.

Locomotor activity and exploratory behavior were tested in a modified hole-board apparatus as described by File & Wardill (1975). It consisted of a dimly illuminated (40 W) circular box (1 m in diameter) with a plexiglass wall. The floor was divided into 10 × 10 cm squares with four equally spaced holes (3 cm dia.) in the central area. Because of the small size of hypothyroid rats, an interchangeable floor was built in which the diameter of the holes was reduced to 1.5 cm. The area under the floor of the apparatus was painted black. Rats were tested during four consecutive days, for 4 min each day, between 1400 hr and 1600 hr. Ambulations (number of squares entered), numbers of rearing and defecations (number of boli), as well as the number of head-dips (scored if both eyes reached the level of the hole) were recorded. The experimental apparatus was cleaned with 70% ethanol after each animal.

To evaluate habituation occurring for each type of activity during the subsequent test days, an Index of Habituation (I.H.) was calculated according to Tamasy *et al.* (1973). The I.H. was expressed as the ratio of activity recorded on the third and fourth test days and the total activity summed over the 4 days of the experiment. An I.H. value lower than 0.5 reflected a decrease of activity in the course of consecutive days. This index has proved to be a reliable indicator of habituation (Tamasy *et al.*, 1973).

Passive avoidance learning (PA) was tested in a light avoidance, one-trial, step-through situation. The experimental box consisted of a well-illuminated (100 W) white and a dark, black, compartment (each 30 × 30 × 30 cm), separated by a wall with a hole (5 × 5 cm). On the training day, rats were placed in the white compartment and allowed to explore the experimental cage for 180 sec; then they were returned to the white compartment, and the latency of step-through was measured. Animals showing an initial step-through latency longer than 60 sec were discarded. In the black compartment, rats received unavoidable electric footshocks (0.3 mA intermittently for 15 sec) through the grid floor, and within 60 sec they were returned to their living cages.

Memory retrieval (PA learning) was tested 24 and 48 hr later for 180 sec, and the latency of reentering the black box was measured.

To investigate the responsiveness of the brain – pituitary – thyroid system, 90-day-old normal and rehabilitated rats were exposed to various types of neural stress using the tests and equipment described previously. Four groups of animals each consisting of approximately half normal and half rehabilitated rats, were exposed to the following situation.

Group I — maintained undisturbed in home cage until taken for immediate sacrifice (non-stressed controls);

Group II — transferred from home cage to hole-board apparatus and maintained there for 10 min after which they were killed (mild stress);

Group III — transferred from home cage to shuttle box with foot shock for 10 min, after which they were sacrificed (pain stress).

Group IV — exposed to foot shock as in Group III, then returned to home cage; 24 hr later returned to shuttle box without foot shock for 10 min, after which they were killed (emotional stress).

Killing was by decapitation, and blood samples were collected immediately for hormonal analysis.

Hormonal analyses

To establish the degree of hypothyroidism induced by our treatment and the capacity for recovery, serum levels of thyrotropin (TSH), thyroxine (T4), and triiodothyronine (T3) in the serum, were measured in 50 day-old control, PTU-treated, and rehabilitated rats, as well as in 90 day-old control and rehabilitated rats. The same procedure was adopted for the stressed animals. In all instances, the animals were killed between 1400 hr and 1500 hr. Trunk blood was collected, allowed to clot at 4°C for 8 hr, and centrifuged. Sera were stored at – 20°C. For each hormone, all samples were run in duplicate in a single assay. For each hormone assay, the maximum intra-assay variability was less than 15%.

TSH was measured by double antibody radioimmunoassay (RIA) with the kit obtained from the Rat Pituitary Hormone Program of NIADDK. Data were expressed in terms of Rat TSH Reference RP-2 (AFP-5153B). Serum levels of T4 and T3 were determined by RIA technique based on the method of Chopra (1972) and Chopra *et al.* (1972).

Statistical analysis

Statistical analysis was performed by Student's *t*-test, non-parametric Mann – Whitney U-test, and one-way analysis of variance (ANOVA) followed by *t*-test for between-group comparisons.

RESULTS

Growth and body weight gain

As shown in Figures 1 and 2, body weight gain was severely depressed in the PTU-treated rats. After weaning (day 25) weight gain in the hypothyroid rats was negligible. Withdrawal of PTU resulted in resumption of active growth, but complete compensation of the growth deficit did not occur.

Serum concentrations of TSH and thyroid hormones

In 50-day-old PTU-treated rats and eight-fold increase of TSH serum concentration was associated with non-detectable T4 and a low level of T3 (Table I). Compared to the control group, the serum T3 levels in the rehabilitated 50-day-old rats were normal; serum TSH levels were lower but not significantly so; and the T4 concentration was still significantly lower. T4 and T3 levels of rehabilitated rats at 90 days of age were normal (Table I); their serum TSH level was still lower, though not significantly so.

Responsiveness of thyroid gland to neural stress

At 90 days of age rats were exposed to various types of neural stress for 10 min. In normal rats, exposure to novel environment (mild stress) did not alter T3 and T4 levels. After emotional stress (conditioned fear reaction), T4 was unchanged, but T3 was significantly increased. Painful stimulation (unavoidable electric footshocks) resulted in a marked decrease of circulating T4 and a marked increase in T3 (Figure 3). By contrast, rehabilitated rats exhibited the reverse pattern of changes; T4 increased significantly during painful stimulation (150%), while T3 decreased in response to both pain (62%) and emotional stress (57%).

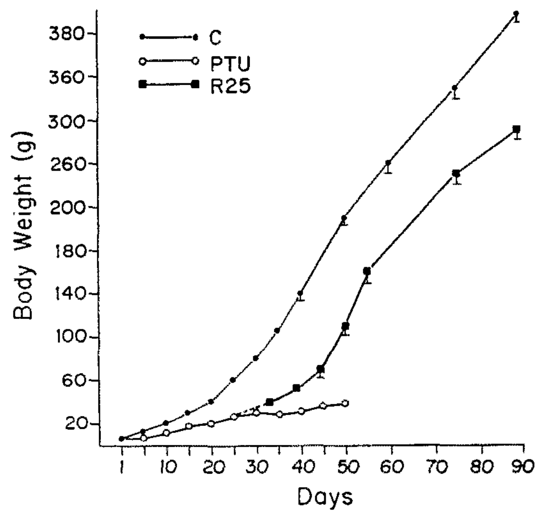

FIG. 1. Body weights of untreated control (C), hypothyroid (PTU), and neonatally hypothyroid rats rehabilitated beginning on day 25 (R25). In each group the weights are the means of 32 – 40 rats. The brackets indicate the S.E.M.

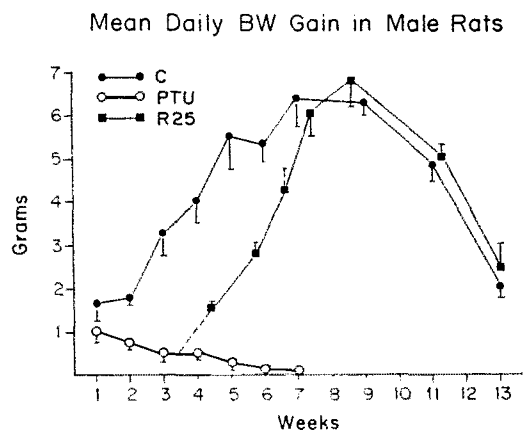

FIG. 2. Mean daily body weight gain of untreated control (C), hypothyroid (PTU), and neonatally hypothyroid rats rehabilitated beginning on day 25 (R25). In each group the weights are the means of 32 – 40 rats. The parentheses indicate the S.E.M.

Spontaneous motor activity

Between the ages of 42 and 50 days each group of animals exhibited a circadian rhythm

TABLE I. PITUITARY – THYROID HORMONE CONCENTRATIONS IN THE SERUM OF MALE RATS

Treatment	TSH (ng/ml) 50 (days)	TSH (ng/ml) 90	T4 (μg/100ml) 50 (days)	T4 (μg/100ml) 90	T3 (ng/100ml) 50 (days)	T3 (ng/100ml) 90
Control	1.86 ± 0.27*	1.70 ± 0.25	5.67 ± 0.25	5.86 ± 0.76	89.32 ± 8.45	79.25 ± 9.70
Hypothyroid (PTU)†	15.30 ± 1.36§	—	ND‡	—	33.58 ± 5.89§	—
Rehabilitated (PTU withdrawn on day 25)	1.25 ± 0.39	1.33 ± 0.28	4.24 ± 0.50§	5.84 ± 0.62	86.88 ± 8.85	81.53 ± 12.49

*Mean ± S.E.M. for 11 – 16 animals per group.
†Each sample was pooled blood from three rats due to small body size.
‡Not detectable. (Limit of assay, 0.5 μg/100 ml).
§$p < 0.05$ (Student's *t*-test).

of spontaneous motor activity in the running-wheel apparatus (Figure 4A). Hypothyroid rats showed activity only at the beginning of the dark period between 2000 hr and 2200 hr, and their average 24-hr activity score was 14% of that of the controls (122.0 ± 31.24 vs 879.40 ± 55.31, $p < 0.01$). The 42- to 50-day-old rehabilitated rats were clearly hyperactive

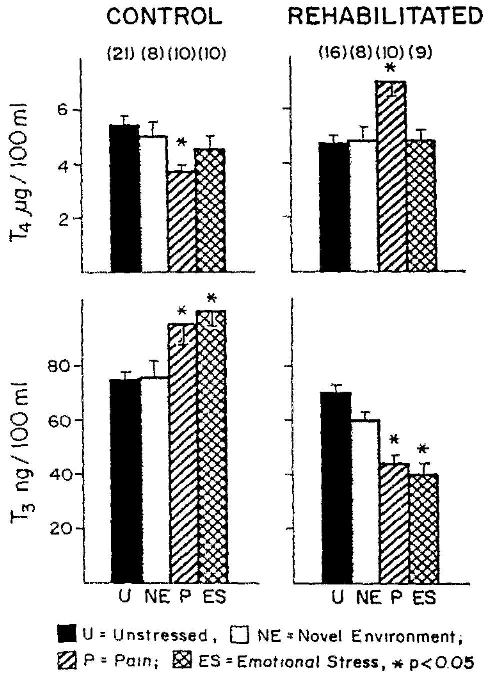

FIG. 3. Changes in serum concentrations of T4 and T3 in 90-day-old rats in response to various types of neural stress. In each group the number of rats is shown in parentheses above the bars. The barogram represents the mean, and the brackets indicate the S.E.M.

in this behavioral test (2255.0 ± 150.50, $p < 0.01$), though distribution of their activity in part resembled that of the normal males. Thus, the pattern of spontaneous motor activity over the light – dark cycle in the rehabilitated rats, which exhibited two peak values between 2000 hr and 2200 hr and 0400 hr and 0500 hr was similar to that of the controls, except for an additional intermediate peak in the rehabilitated rats, occurring between 2400 hr and 0100 hr, which was not observed in the normal rats. By day 90, spontaneous motor activity of rehabilitated rats had decreased greatly, but it was still significantly higher than that of the controls (1306.25 ± 36.82 vs 767.97 ± 97.89, $p < 0.01$). (Figure 4B). The pattern of activity at this age was, however, identical to that of the controls (Figure 4B).

Locomotor activity, exploratory behavior and habituation

Rats were exposed to a novel environment in a modified hole-board apparatus for 4 min over four consecutive days. ANOVA test revealed a significantly increased locomotor activity in the 50-day-old rehabilitated rats ($F(2,43) = 5.98$, $p < 0.05$), while exploratory activity for the PTU-treated and rehabilitated groups, as reflected by the rearing and hole-dipping scores, was significantly lower than that of the normal controls: $F(2,43) = 4.80$, $p < 0.05$; and $F(2,43) = 5.28$, $p < 0.05$, respectively (Table II). In the early phase of rehabilitation (days 42 – 50), rehabilitated rats had significantly higher defecation scores than controls ($F(2,43) = 3.72$, $p < 0.05$). At 90 days of age, the increased locomotor activity of the rehabilitated rats still persisted ($F(1.21) = 14.32$, $p < 0.01$), while their exploratory behavior and defecation did not differ significantly from those of normal rats (Table II).

TABLE II. HOLE-BOARD SCORES OF MALE RATS RECORDED OVER FOUR CONSECUTIVE DAYS

	Locomoter activity		Rearing		Hole-dipping		Defecation	
Treatment	50 (days)	90	50 (days)	90	50 (days)	90	50 (days)	90
Control	298.90 ±10.35*	310.25 ±15.36†	51.17 ±2.38	62.18 ±4.25	37.61 ±2.05	40.22 ±3.33	14.92 ±1.22	16.8 ±2.42
Hypothyroid (PTU)	316.83 ±17.29	—	31.65 ±1.86‡	—	16.64 ±0.95‡	—	8.17 ±.056‡	—
Rehabilitated (PTU withdrawn on day 25)	413.99 ±16.32‡	428.97 ±22.48‡	37.06 ±2.02‡	58.35 ±4.74	28.91 ±1.46‡	37.88 ±3.83	19.82 ±1.40‡	17.60 ±1.98

*Mean ± S.E.M. for 18 to 22 male rats tested between 42 and 50 days of age.
†Mean ± S.E.M. of 10 to 13 male rats tested between 90 and 100 days of age.
‡$p < 0.05$; significantly different from control.

In both age groups (50 and 90 days), control rats showed habituation to the novel environment in each behavioral parameter measured (Table III). Both 50-day-old PTU treated and the 50-day-old rehabilitated animals exhibited a decrease of locomotor activity over the 4 days of testing, but no habituation of rearing, hole-dipping or defecation. However, by 90 days of age, the rehabilitated rats were capable of habituating to the novel environment to the same extent as the control animals (Table III).

TABLE III. INDEX OF HABITUATION* IN MALE RATS

Treatment	Locomoter activity 50 (days)	90	Rearing 50 (days)	90	Hole-dipping 50 (days)	90	Defecation 50 (days)	90
Control	0.452 ±0.008†	0.360 ±0.003‡	0.460 ±0.003	0.475 ±0.007	0.424 ±0.009	0.436 ±0.008	0.486 ±0.006	0.450 ±0.009
Hypothyroid (PTU)	0.445 ±0.007	—	0.545 ±0.005§	—	0.602 ±0.007§	—	0.613 ±0.012§	—
Rehabilitated (PTU withdrawn on day 25)	0.441 ±0.007	0.395 ±0.004	0.536 ±0.003§	0.465 ±0.006	0.684 ±0.013§	0.420 ±0.007	0.525 ±0.010§	0.487 ±0.010

*The Index of Habituation is a ratio of the activity recorded on days 3 – 4 to the total activity over 4 days. Index lower than 0.5 refers to decrease of activity.
†Mean ± S.E.M. for 18 – 22 male rats tested between 42 and 50 days of age.
‡Mean ± S.E.M. for 10 – 13 male rats tested between 90 and 100 days of age.
§No habituation occurred over the 4 test days.

Passive avoidance learning

Analysis of one-trial passive avoidance learning in a step-through situation revealed no significant differences among control, PTU-treated, and rehabilitated 50-day-old rats, either in the initial latency to step-through on the training day (control = 33.07 ± 6.60 sec; PTU = 25.0 ± 4.33 sec; rehabilitated = 29.28 ± 5.28 sec), or in the memory consolidation and/or retrieval 24 and 48 hours after the learning trial (Mann – Whitney U-test, $p > 0.1$) (Figure 5).

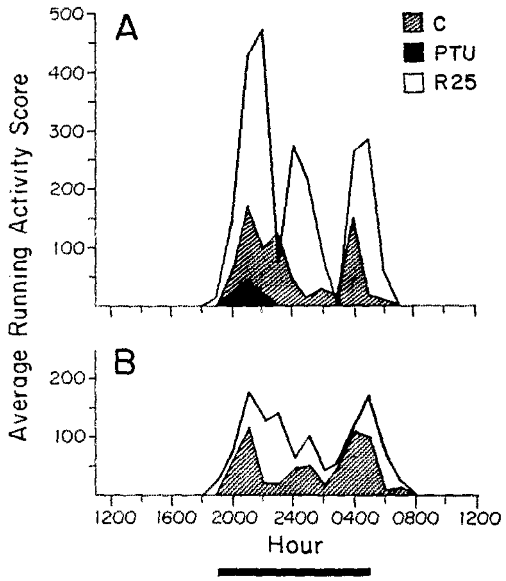

FIG. 4. Circadian distribution of spontaneous locomotor activity of 42 – 50-day-old (A) and 90 – 100-day-old (B) male rats measured in a running-wheel. Data are expressed as average count/hr recorded on 4 consecutive days. C: controls; PTU: hypothyroid; R25: neonatally hypothyroid rats rehabilitated beginning on day 25. The horizontal black bar indicates the dark time of day.

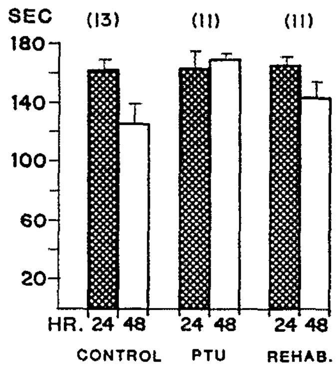

FIG. 5. Passive avoidance learning of normal (CONTROL), hypothyroid (PTU), and rehabilitated (REHAB) rats. Memory retention was tested 24 and 48 hr after the one-trial learning session and is expressed by latency (sec), before animals re-entered the black compartment. Number of rats is shown in parentheses. The barogram represents the mean, and the brackets indicate the S.E.M.

DISCUSSION

Hypothyroidism during the postnatal period, induced by the administration of the goitrogen PTU, is known to retard markedly body growth and maturation as well as development of the nervous system and behavior. We previously have shown that the administration of 0.1% PTU in the drinking water of the mother, as in the present study, increased blood TSH levels fourfold in the mothers and tenfold in the pups and completely supressed serum T4 levels in both (Tamasy *et al.*, 1984). The eightfold increase in TSH associated with complete suppression of T4 in the 50-day-old hypothyroid rats in the present study agrees with our previous findings. The continued presence of significant amounts of T3 in the blood of these hypothyroid rats (30% of normal) cannot be explained unless it was due to an artifact of the immunologic assays, as the animals showed all the symptoms of hypothyroidism and experimental cretinism and had no T4 in their blood. This unusual presence of T3 in the blood of essentially athyroid rats also has been reported by other investigators (Mosier, 1977).

Although PTU withdrawal eventually resulted in normalization of the plasma levels of the hormones by day 90, the rate of recovery for the various hormones was different, T3 showing a faster and T4 a slower recovery. We do not know whether the tendency of TSH to be lower (non-significantly) in the rehabilitated animals is the result of low sample numbers or has any special significance. However, normal TSH and depressed T4 levels in the serum of young adult rats treated with PTU in infancy has been reported by others (Poland *et al.*, 1983).

In contrast to the normalization of base-line levels of plasma T3 and T4, our results indicate significant differences between the control and rehabilitated animals in the stress-induced changes in hormone levels. However, in the absence of relevant data in the two groups of animals concerning the levels of TSH and TRH as well as the activity of metabolizing enzymes of T3 and T4 in the periphery, the relative contribution of central regulatory factors vs peripheral factors (i.e., changes in volume of distribution of these hormones in the plasma and tissues or their metabolic enzymes, etc.) in the genesis of the above differences cannot yet be determined. Various types of stress (bleeding, etherstress, restraint) decrease TSH secretion and circulating levels of T4 (Simpkins *et al.*, 1978); however, an increase in serum T3 without a significant change in T4 was reported following "disturbance stress" (Dohler *et al.*, 1977). Our observations that exposure of normal rats to mild stress does not affect T3 or T4 levels, while painful stimulation elicits marked depression of serum T4 within 10 min, and that both pain and emotional stress elevate T3 levels, agree with the earlier data. A significant finding of the present experiment is that in rehabilitated rats exposed to pain and emotional stress, changes in serum T4 and T3 levels are opposite to those which occur in control males. The causes of these differences and their peripheral or central origin require further investigation. It should be noted that neonatal thyroid deficiency is known to disturb the secretion of other pituitary hormones as well (Kikuyama *et al.*, 1974; Tamasy *et al.*, 1984; Takeuchi *et al.*, 1978; Wong *et al.*, 1980); these other changes may influence the behavior of the hypothyroid and rehabilitated animals.

The marked improvement in body growth and somatic maturation in the rehabilitating rat, as well as the failure of the male rats to show complete compensation of growth deficit, is in agreement with a more detailed study of this subject (Meisami, 1984).

Our behavioral observations in the hypothyroid and rehabilitated rats indicate that neonatal thyroid deficiency has different effects on the development of various types of behavior. Thus, simple memory processes—e.g., passive avoidance learning—were not influenced by the hypothyroid state, while other adaptive behavioral reactions—e.g., exploration, habituation, and emotionality (reflected by defecation (Denenberg, 1969)—were significantly altered by hypothyroidism but were restored to normal with rehabilitation. Yet other behavioral activities—e.g., spontaneous motor activity in the running-wheel and locomotor activity in the hole-board tests—were significantly increased in the rehabilitated rats compared to the controls. This hyperactivity, though markedly reduced, still persisted 2 months after the beginning of rehabilitation, suggesting the need for longer periods of rehabilitation.

Morphological and biochemical studies indicate that the development of various CNS areas are affected differentially by neonatal thyroid deficiency. Thus, while in the cerebral cortex mainly the differentiation of the neurons and glia is adversely affected, in other structures such as cerebellum, olfactory bulbs, and dentate gyrus, neurogenesis, gliogenesis, synaptogenesis, and differentiation of neuronal processes are altered. Indeed, even the more primitive structures of the CNS (such as the spinal cord), with an earlier timetable of maturation, are not spared by thyroid deficiency (Balazs *et al.*, 1971; Eayrs, 1964; Ford & Cramer, 1977, Grave, 1977; Lauder, 1983; Legrand, 1983; Meisami, 1979; Meisami, 1983; Timiras & Luckock, 1974). Ontogenetic development of spontaneous locomotor activity and several adaptive behaviors are believed to be associated with maturation of the hippocampal formation (Leblanc & Bland, 1979). In earlier electrophysiological studies we reported that, in the rat hippocampus, characteristic EEG waves as well as neuronal inhibitory functions appear between the 12th and 15th postnatal days (Tamasy *et al.*, 1977, 1980). Several investigators (Rabie *et al.*, 1979; Lauder & Mugnaini, 1980; Lauder, 1983; Lipp *et al.*, 1984) have demonstrated that the development of the hippocampus is impaired in hypothyroidism, with little prospect for recovery.

Our findings of a lack of habituation in hypothyroid rats, as well as the persistence of increased locomotor activity (spontaneous or in response to novel environment) in the rehabilitated rats, suggest increased excitability of the CNS which, among other factors, may be associated with impaired or retarded development of the inhibitory neuronal circuits of the hippocampus and other forebrain structures. This possibility is in agreement with the earlier investigation by Meisami *et al.* (1970) in which a significantly reduced electroshock seizure threshold in one-month-old hypothyroid rats also was interpreted as increased brain excitability due to impaired development of inhibitory mechanisms.

With regard to the generally promising picture of rehabilitation seen here, several points need emphasis. Earlier research on thyroid and developing brain-behavior had focused on the deficiencies of the cretinoid rat, perhaps as a model for human mental retardation (Eayrs, 1966; Hamburgh *et al.*, 1964; Grave, 1977). Indeed, it often is stated that early hypothyroidism is associated with permanent and irreversible behavioral and mental retardation, even though Eayrs' research had indicated normalization or marked improvements in several tests, except the Hebb – Williams test, with postweaning hormonal replacement therapy.

The present results leave little doubt that the prospects of behavioral recovery from early hypothyroidism are better than commonly assumed. Not only do hypothyroid rats perform as well as controls in some tasks (passive avoidance learning/memory retentions), but when deficient (as in exploratory behavior, habituation, and locomotor activity), they can show complete recovery or marked improvement of behavior with normalization of thyroid function and consequent somatic recovery. Indeed, even in the instance of incomplete normalization of running-wheel activity, the animals scored much closer to normal 2 months after the beginning of rehabilitation than after 1 month, suggesting that the difference may eventually disappear with a longer rehabilitation time. We also may infer from these results that the plasticity of the developing brain, particularly in relation to behavior, may extend over a longer period than commonly assumed. Indeed, the notion of a fixed critical period in the preweaning phase for thyroid effects on brain – behavior development [to which we ourselves subscribed (Timiras, 1972)] may require some re-evaluation.

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